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Advanced magnetic resonance spectroscopy and imaging techniques applied to brain development and animal models of perinatal injury

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ABSTRACT

Magnetic resonance spectroscopy (MRS) and magnetic resonance imaging (MRI) are widely used in the field of brain development and perinatal brain injury. Due to technical progress the magnetic field strength (B_0) of MR systems has continuously increased, favoring ^{1}H-MRS with quantification of up to 18 metabolites in the brain and short echo time (TE) MRI sequences including phase and susceptibility imaging. For longer TE techniques including diffusion imaging modalities, the benefits of higher B_0 have not been clearly established. Nevertheless, progress has also been made in new advanced diffusion models that have been developed to enhance the accuracy and specificity of the derived diffusion parameters. In this review, we will describe the latest developments in MRS and MRI techniques, including high-field ^{1}H-MRS, phase and susceptibility imaging, and diffusion imaging, and discuss their application in the study of cerebral development and perinatal brain injury.

1. Introduction

Due to technical advances, the static magnetic field (B_0) of magnetic resonance (MR) systems has continuously increased. Clinical state-of-the-art scanners reach 3.0 T, although 7.0 T and 9.4 T are also available for clinical research, while animal scanners range from 3.0 T to 21.0 T, including 4.7 T, 7.0 T, 9.4 T, 11.7 T, 14.1 T and 17.0 T. This drive for increasing B_0 relates to the almost linear increase in the signal-to-noise ratio (SNR) with B_0, due to an almost linear increase of magnetization of the sample (Callaghan, 1991). This increase in SNR provides increased image resolution and reduced scanning time (*i.e.*, "better and faster").

An increase in the spectral resolution with B_0 is particularly advantageous for MR techniques such as MR spectroscopy (MRS). The recent development of advanced localized ^{1}H magnetic resonance spectroscopy (^{1}H-MRS) at high magnetic fields (*e.g.*, ≥7.0 T) has allowed quantification of concentrations of up to 18 metabolites in the rodent brain (termed the "neurochemical profile"), including antioxidants, compounds related to energy metabolism, neurotransmission, membrane precursor, osmoregulation, myelination, neuronal markers, glial markers, and neuroprotection (Lei et al., 2009; Mlynarik et al., 2008, 2006; Tkac et al., 2003; van de Looij et al., 2011). ^{1}H-MRS has also been used to follow the changes in the neurochemical profile during rat brain development and maturation (Tkac et al., 2003). Further, cerebral hypoxia-ischemia (HI) or inflammation leads to specific changes in the neurochemi-

Abbreviations: Mac, macromolecules; Asc, ascorbate; bhB, beta-hydroxibutirate; PCho, phosphorylcholine; Cr, creatine; PCr, phosphocreatine; GABA, γ-aminobuttyric acid; Glc, glucose; Glu, glutamate; Gln, glutamine; myo-Ins, myo-inositol; Lac, lactate; NAA, *N*-acetylaspartate; NAAG, *N*-acetylaspartylglutamate; PCr, phosphocreatine; PE, phosphoethanolamine; Tau, taurine; HI, hypoxia-Ischemia; LPS, lipopolysaccharide; MRI, magnetic resonance imaging; MRS, magnetic resonance spectroscopy; SNR, signal-to-noise ratio; TE, echo time; DTI, diffusion tensor imaging; QSM, quantitative susceptibility mapping; ADC, apparent diffusion coefficient; MD, mean diffusivity; $D_{//}$, parallel diffusivity; $D_{\perp}$, orthogonal diffusivity; FA, fractional anisotropy; NODDI, neurite orientation dispersion and density imaging; ficvf, intra-neurite volume fraction; fiso, cerebrospinal volume fraction; fia, intra-axonal volume fraction; ODI, orientation dispersion index; MEMRI, manganese enhanced magnetic resonance imaging; DKI, diffusion kurtosis imaging; MK, mean kurtosis; $K_{//}$, parallel kurtosis; $K_{\perp}$, orthogonal kurtosis; fMRI, functional magnetic resonance imaging.

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cal profile, especially in the acute phase (Lodygensky et al., 2014; van de Looij et al., 2011, 2014a,b; Xu et al., 2014). Thus, ^{1}H-MRS appears to be particularly suitable for assessing longitudinal brain development and efficacy of neuroprotective strategies following perinatal brain injury, as well as for providing data that can be easily translated to humans.

For MR imaging (MRI) techniques utilizing short echo times (TE) such as phase imaging, the gain in signal obtained with a higher B_0 combined with an increased frequency shift distribution can provide increased spatial resolution and reduced scanning times (Marques et al., 2009). Since the phase of gradient echo MR images is sensitive to differences in the resonance frequency, it can be used to create anatomical images with superb contrast between white matter and grey matter at high magnetic fields (Marques et al., 2009). More recently, the development of magnetic quantitative susceptibility mapping (QSM), derived from phase images, has allowed quantitative contrast, which is not the case with phase contrast (de Rochefort et al., 2010). While the origin of phase contrast is not fully understood, it is related to the magnetic susceptibility of the tissues. Several putative assumptions have been made, including an effect of iron, myelin or lipid content, as well as an effect of fiber orientation compared to the main magnetic field strength or packing of axons (Wharton and Bowtell, 2015). Phase contrast imaging has been recently used to assess the myelination process in the developing mouse brain, making it a powerful tool to assess myelination deficits, a hallmark of preterm brain injury (Lodygensky et al., 2012).

The signal gain obtained by increasing B_0 can be further enhanced with the use of contrast agents such as manganese (Mn^{2+}) known to reduce T_1 relaxation time, leading to enhanced signal of T_1W images in regions of Mn^{2+} accumulation, termed manganese-enhanced MRI (MEMRI) (Koretsky and Silva, 2004). Indeed, MEMRI has been shown to be a powerful tool to delineate long-term injury following mild HI in the developing rodent brain (Yang et al., 2008; Yang and Wu, 2008).

Diffusion tensor imaging (DTI) measures the movement of water in the tissue, and can provide useful information on fiber direction (Basser et al., 1994a,b) or integrity (Beaulieu et al., 2002) in the white matter. As such, DTI has been widely used to study brain development and perinatal brain injury in humans (Ment et al., 2009) and animals (van de Looij et al., 2014d). In contrast to MRS and short-TE MR techniques, the benefits of increasing B_0 are more controversial for long-TE techniques. Indeed, when using a higher magnetic field, the apparent transverse relaxation time T_2 will also be shortened (Kunz et al., 2013; Marques et al., 2009). Therefore, the gain in SNR may be attenuated for techniques that use long TEs such as diffusion imaging, where TE is constrained by the duration of the diffusion gradients. In addition, the magnetic susceptibility effects are larger at higher fields, constraining rapid imaging that is mandatory for *in-vivo* studies. Thus, for imaging modalities such as diffusion imaging, the benefits of an ultra-high B_0 remain unclear. For instance, Kunz et al. found no difference in the SNR of DTI images at 9.4 T and 14.1 T (Kunz et al., 2013). The gain in SNR attributed to higher B_0 is counterbalanced by the decrease of T_2 relaxation time from 9.4 T to 14.1 T, leading to a decrease of the SNR.

For diffusion imaging, B_0 is not the only source of progress, with recent development of new diffusion models. The main limitation of conventional DTI is that the parameters derived from DTI (mean diffusivity, MD; parallel diffusivity, $D_{//}$; orthogonal diffusivity, $D_{\perp}$; fractional anisotropy, FA) are sensitive, but not-specific, to the tissue microstructure. For instance, anisotropy can be modulated by the degree of myelination, axonal density, axon diameter distribution, orientation coherence and cell membrane permeability (Barazany et al., 2009; Favrais et al., 2011; Huppi and Dubois, 2006; Nair et al., 2005; Sizonenko et al., 2007b; van de Looij et al., 2012a,b). Several models have been proposed to improve specificity of diffusion derived parameters such as diffusion kurtosis imaging (DKI) (Jensen and Helpern, 2010) or multi-compartment models (Alexander et al., 2010; Assaf and Basser, 2005; Assaf et al., 2008; Barazany et al., 2009; Zhang et al., 2012), with some already applied to assess brain development. DKI is an extension of DTI, which includes non-Gaussian diffusion assessment and has been used to follow brain development in human (Paydar et al., 2014) and rodents (Cheung et al., 2009). Recently, the neurite orientation dispersion and density imaging (NODDI (Zhang et al., 2012)), a new practical diffusion MRI technique for estimating the microstructural complexity of neurites (*i.e.,* dendrites and axons) has been successfully used *in-vivo* on clinical MRI scanners (Kunz et al., 2014; Zhang et al., 2012). This model can be used to estimate the intra-neurite volume fraction (*ficvf*), the cerebrospinal volume fraction (*fiso*) and a new index called orientation dispersion index (ODI) to model the dispersion/fanning of the axonal fibers or dendrites. This approach appears to be promising in the study of grey matter changes during development and injury.

Finally, a major advantage in the field of MR in perinatal brain injury is application of multimodality, involving the combination of neurochemical (by MRS) and/or anatomical (by MRI and/or MEMRI) and microstructural (by DTI and/or DKI and/or phase imaging, QSM) information non-invasively, which allows accurate, real time assessment of brain development and mechanisms and consequences of perinatal brain injury.

The aim of this review is to discuss how progress in MRS/MRI techniques can be used in the study of brain development, perinatal brain injury and neuroprotective strategies. We will also identify various high-field MRS and MRI modalities applied in animal studies that may have human clinical applications. The review is organized by MR techniques enhanced by high magnetic field (^{1}H-MRS and short TE MRI techniques), advanced diffusion imaging techniques, and multimodality.

2. High-field ^{1}H-magnetic resonance spectroscopy

The ability of *in-vivo* short echo-time proton ^{1}H MRS to determine the concentrations of up to 18 metabolites in rodent brain (Pfeuffer et al., 1999) allows detailed assessment of multiple neurochemical markers in animal models of perinatal brain injury. High magnetic field increases the accuracy and precision of metabolite quantification due to higher SNR combined with increased spectral differentiation. This is particularly beneficial for quantification of low concentration metabolites or metabolites with overlapping spectral lines.

Short TE sequences such as ultra-short echo time SPECIAL spectroscopy (Mlynarik et al., 2008) have also been developed to enhance SNR. Using this sequence, up to 18 metabolites were quantified in human brain at 3.0 T and 7.0 T (Mekle et al., 2009), as well as in the rodent brain at 9.4 T and 14.1 T (Lei et al., 2009; Mlynarik et al., 2008, 2006; Tkac et al., 2003; van de Looij et al., 2011) (Table 1 and Fig. 1). Several of these metabolites were particularly relevant in the context of perinatal brain injury following HI and inflammation. Lactate (Lac) is the end product of anaerobic glycolysis. The very low concentration of Lac in the normal brain makes it difficult to quantify by ^{1}H-MRS, although Lac rapidly increases in clinical situation of HI and/or inflammation (Rudkin and Arnold, 1999). Experimentally, a high level of Lac was found in cortical lesions of pup rats at 24 h post HI (van de Looij et al., 2011), likely related to secondary energy failure and mitochondrial dysfunction (Penrice et al., 1997; Vannucci et al., 2004), although production of Lac by phagocytic cells infiltrating the brain may also contribute (Lanfermann et al., 1995). A similar increase of Lac was found at 24 h after lipopolysaccharide (LPS) injection into the corpus callosum of neonatal rats (Lodygensky et al., 2014).